THE USE OF *Artemia* CYSTS IN AQUACULTURE: THE CONCEPT OF "HATCHING EFFICIENCY" AND DESCRIPTION OF A NEW METHOD FOR CYST PROCESSING

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THE USE OF *Artemia* CYSTS IN AQUACULTURE: THE CONCEPT OF "HATCHING EFFICIENCY" AND DESCRIPTION OF A NEW METHOD FOR CYST PROCESSING

P. Sorgeloos,[1] G. Persoone, M. Baeza-Mesa,
E. Bossuyt and E. Bruggeman
State University of Ghent
Laboratory for Biological Research
in Environmental Pollution
and
Artemia Reference Center
J. Plateaustraat 22
B 9000 Ghent, Belgium

Institute for Marine Scientific Research
Prinses Elisabethlaan 69
B 8401 Bredene, Belgium

ABSTRACT

The quality of brands of *Artemia* cysts should be expressed on a weight basis, namely the number of live nauplii hatching out per unit of weight of product and not as a "hatching percentage" since the latter concept does not take into account the degree of impurity of the material. A standardized method for the determination of the hatching efficiency is proposed. A new methodology for cyst processing has been worked out at laboratory scale, based on a 2-step cleaning (brine and tap water) followed by dehydration (air or brine) or decapsulation and dehydration (brine).

INTRODUCTION

During the annual meeting of the World Mariculture Society in Costa Rica in 1977, an informal brainstorming session was held on the problematics of utilization of brine shrimp in aquaculture. Several interesting facts appeared from the floor discussions:

1. There seems to be no guarantee of the quality of *Artemia* cysts sold commercially.
2. The quality of these cysts can fluctuate greatly from one commercial brand to another and it also varies for the same brand from year to year.

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[1] "Aangesteld Navorser" at the Belgian National Foundation for Scientific Research (N.F.W.O.).

3. No standard method has yet been adopted or worked out to assess the hatchability of a given batch.

4. No intercalibration has ever been performed to compare brands of different origin.

As the *Artemia* research team of our laboratory (presently established as "*Artemia* Reference Center") has been working for many years on the problematics of the utilization of brine shrimp in aquaculture, we thought it our duty to try at least to solve some of the problems just mentioned.

THE CONCEPT OF "HATCHING EFFICIENCY"

Until now the hatchability of a given sample of cysts has always been expressed as hatching percentage, namely the number of live nauplii hatching out of 100 cysts. This criterion, however, does not take into account the degree of purity of the product; in other words, it does not consider the quantity of debris included in the batch of cysts. In this regard the concept of hatching percentage is greatly misleading since a figure of 90% hatching may indeed be correct despite the fact that the product may carry an incredible quantity of debris, so that on a weight basis only a very low percentage of the commercial brand really consists of full cysts.

If relative comparisons can be made from one batch to another on brands originating from the same geographical locale, which have been submitted to the same processing technique, comparison of cyst batches from different salinas or salterns is much more hazardous; indeed, the dimensions of the cysts can vary notably from one geographical strain to another (Claus et al., 1977); also the harvesting and processing techniques applied can differ considerably. Since more and more commercial brands are appearing on the market, a simple, routine, standardized method for evaluating the quality of *Artemia* cyst brands is highly desirable.

Inasmuch as *Artemia* cysts are always sold on a weight basis the criterion of first importance to the customer is, of course, the number of live nauplii he will get from the total quantity of product purchased. We have worked out the following simple technique to determine the weight of product, which under standard conditions of incubation will yield one million nauplii (Fig. 1).

Three samples of 250 mg per sample are taken at random from the batch of cysts to be analyzed. The cysts are hydrated in cylindroconical tubes in 80 ml natural seawater, at 30°C, and kept in suspension by continuous aeration from the bottom of the tube. After one hour the water volumes are adjusted to 100 ml with seawater and 5 subsamples of 250 µl each are taken from each tube with an automatic micropipet. Each subsample is pipetted in a plastic tube, the microtip of the pipet is flushed with seawater and the water level in the tube is brought to 4 ml with seawater (since at the end of the hatching period all nauplii are counted in each tube, the volume of seawater in the tubes may vary in function with the type of tube used). The tubes are closed with a cap and clamped into a rotating axle at 5 rpm. The whole setup is incubated at 30°C in continuous light conditions.

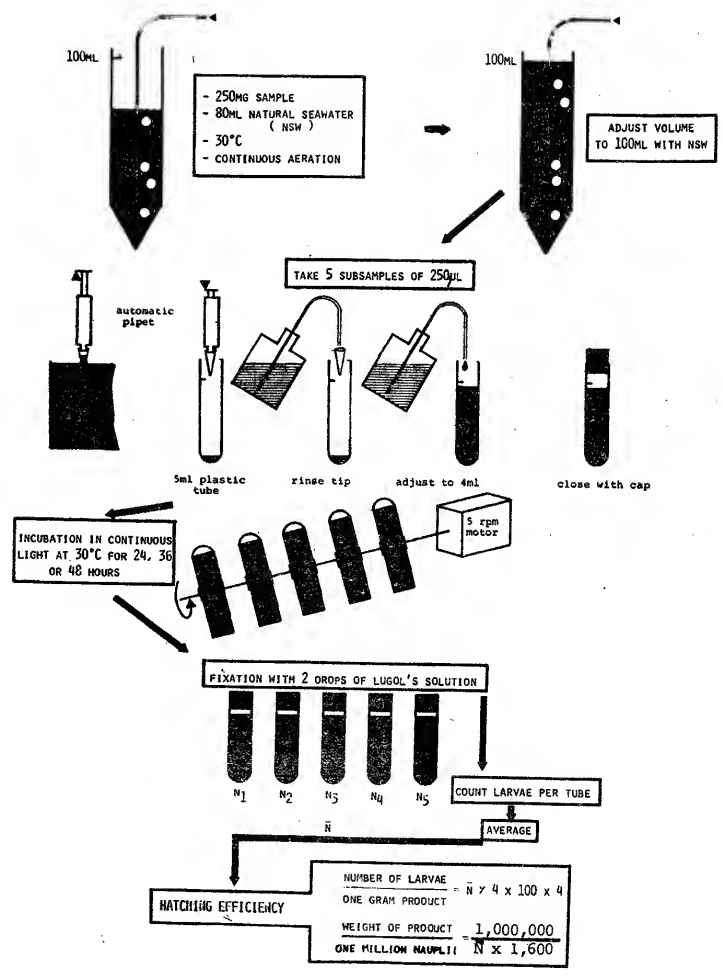

Figure 1. Standard method for the determination of the hatching efficiency of *Artemia* cyst batches.

After 48 hours, the content of each tube is fixed by addition of a few drops of lugol's solution which stains the nauplii dark. The total number of nauplii hatched in each tube is determined by filtering the suspension on a small gauze filter, placing the filter in a petri dish and counting the nauplii under a dissection microscope. The average number of nauplii produced per gram of cyst-product is then calculated. This number can also be expressed more practically as the quantity of product that has to be incubated to produce one million nauplii.

A NEW METHOD FOR CYST PROCESSING

As stated during the *Artemia* round table at the WMS meeting in Costa Rica with regard to the quality of commercially available *Artemia* cysts, the least that can be said is that the purchaser of commercial *Artemia* cysts is often also buying a lot of debris. In other words, the cleaning of the rough cyst material is often not adequate and could be improved considerably. We have been studying this problem extensively in our laboratory and we have worked out, at laboratory scale, a procedure which we find most satisfactory (Fig. 2).

The separation of the debris from the full cysts is performed in 2 steps: when the cyst material is suspended in brine, the heavy particles (sand, skeletons, etc.) will sink and the cysts will float. An intermittent aeration from an airtube, at a certain distance from the bottom, improves the separation of the clumps of cysts. The 5-25 min sequence of aeration/non-aeration is continued for about 24 hours. The layer of floating cysts is then skimmed off and the cysts washed thoroughly with tap water on a 200 µ screen.

The elimination of the light debris is carried out in tap water and takes only about half an hour; the full cysts sink to the bottom, whereas the empty cyst shells, plumes, etc., float on the surface. For some brands it is necessary to agitate the surface, e.g., by air-bubbling, to improve the separation. As far as the further processing of the cysts is concerned, we would like to draw attention to 2 other methods for storing *Artemia* cysts, as an alternative to the classic method of drying in air, followed by packaging in vacuum-sealed or nitrogen-flushed cans (Helfrich, 1973).

We found that cysts can be stored very well in hyperosmotic solutions, the simplest form of which is brine made up with technical salts. Since at very high salinity the solubility of gases is zero, the viability of the dehydrated embryos cannot be affected by free radicals which are formed only in the presence of oxygen (Crowe, 1971; Crowe and Clegg, 1973).

After the harvest of the full cysts at the end of the second separation step, there are thus 3 possibilities for further processing:

1. The classic method of air drying and packaging in vacuum-sealed or nitrogen-flushed cans (cf. Helfrich, 1973).
2. Dehydration of the cysts in a solution of 300 g technical NaCl per liter tap water. At room temperature this process takes about 3 to 5 hours. An important point in this regard is that the cysts should be kept in continuous suspension during the treatment in order to assure a complete exposure of the surface of each cyst to the brine. Once the dehydration is complete the volume of brine can be decreased to a minimum and the product packed in watertight containers.

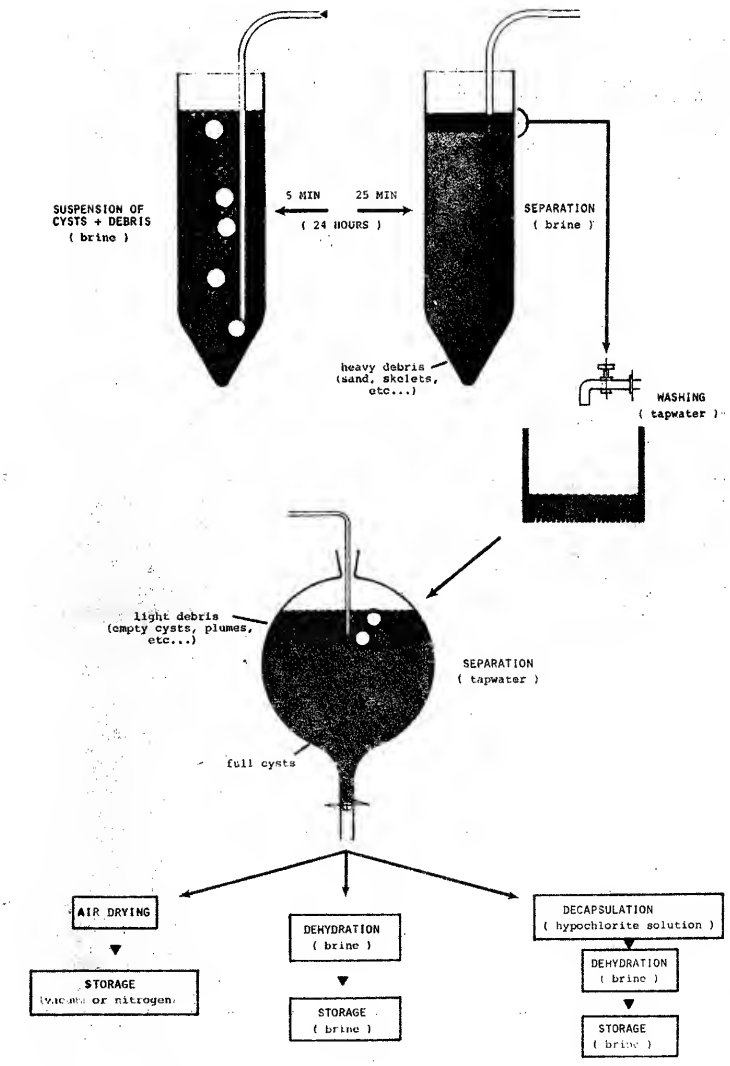

Figure 2. Methodology for cleaning and processing of *Artemia* cysts.

3. Decapsulation of the cysts in a technical hypochlorite solution following a methodology which we described recently (Sorgeloos et al., 1977; Bruggeman et al., in press). This technique suggests a number of interesting perspectives for the practical use of *Artemia* cysts in aquaculture; indeed, it removes the metallochitinic shell usually covered by bacteria and other contaminants, without affecting the viability of the embryo. At the end of the treatment the embryos have a pale rose look, since they are surrounded only by a thin and transparent membrane, but they are perfectly viable. The further processing of these decapsulated cysts is the same as for non-treated material. Upon dehydration in brine the decapsulated cysts take on a coffee-bean shape and sink to the bottom. As the embryos in decapsulated cysts seem to be sensitive to sunlight they should be packed in nontransparent containers.

QUALITY ANALYSIS OF FIVE COMMERCIAL *Artemia* CYST BRANDS OF DIFFERENT GEOGRAPHICAL ORIGIN

The percentage composition of the material purchases is expressed on a dry weight basis with 4 components: the full cysts, the heavy debris (made up of sand, salt, skeletons, etc.), the light debris (such as empty cysts, plumes and other light particles), and water content. The non-treated product has been submitted to our standard hatching technique and the weight of non-cleaned commercial product which has to be incubated to deliver one million live nauplii has been calculated.

On the other hand, hatching tests were performed on the full cysts obtained at the end of the cleaning method and the percentage of cysts which will hatch was determined. The results for 5 commercial brands of different geographical origin are shown in Table 1. The first 3 series are 3 batches from the same trade mark but of different age (collected at different periods).

Table 1. Results of the Analysis of 5 Commercial *Artemia* Cyst Brands

Cyst Supplier	Percentage Composition				Hatching Efficiency		Average Size of Instar I Nauplii (µ)
	Water	Heavy Debris[a]	Light Debris[b]	Full Cysts	Full Cysts (%)	Product per Million Nauplii (g)	
Brand A							444
Batch 1	9.7	7.3	6.0	77	87	4.5	
Batch 2	6.3	10.0	7.0	76	90	4.4	
Batch 3	7.7	2.3	5.0	85	96	3.7	
Brand B	15.6	46.5	19.6	18	82	17.0	396
Brand C	4.0	21.1	15.9	59[c]	7.6	56.0	463
Brand D	2.9	6.2	13.5	77	58	10.0	473
Brand E	10.9	14.5	5.2	69	78	6.0	434

[a]Sand, salt, skeletons, etc.

[b]Empty cysts, plumes, other light particles.

[c]The majority of the cysts are cracked but still contain an embryo.

A first statement is that even the best products contain only 75% of full cysts. Second, the quality of the full cysts as far as their hatching is concerned varies markedly from one brand to another with results ranging from 96 to 7.6%. Last, but not least, when considering the quantity of commercial product needed to obtain one million nauplii, it appears that with one particular brand it suffices to incubate 3.7 g, whereas with another batch of the same trade mark, 20% more of the product is necessary which means a 20% increase in the cost.

The comparison of brands of different origin leads to some amazing conclusions: in comparison to the best quality, i.e., the 3.7 g, almost 2 to 15 times more cyst-product is required to obtain the same number of nauplii. The reader can readily extrapolate for himself the cost-benefit implications of this last finding.

With regard to the foregoing and as a protection for producer as well as consumer, the *Artemia* Reference Center is analyzing regularly different brands of *Artemia* cysts from all over the world to determine their quality. For example, the cysts purchased in bulk by the European Mariculture Society as a service to its members are analyzed by the *Artemia* Reference Center which delivers a label of quality that accompanies every can to the purchaser.

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